Absorbance Laboratory: Further Information

In this laboratory the learning objectives are:

1. To be able to apply the Beer-Lambert law in linear regression.
2. To understand instrumentation principles for measurement of absorption intensity.
3. To understand global analysis.
4. To learn about practical applications of lanthanides as spectroscopic probes.

As a practical matter, this laboratory will also inform students about some experimental artifacts. Understanding how to identify and manage artifacts is a crucial experimental skill. For example, there is often an offset in absorption spectra obtained using a photodiode array spectrometer. This means that the "zero" of the spectrum is offset by a small amount, typically < 0.002. Although this is a small shift it is important for any data points with an absorbance < 0.01. The best treatment of this type of offset is to identify a region of the spectrum that is flat and has no absorption bands. Then offset each spectrum in a series by subtracting a constant so that each spectrum has zero absorbance over the selected range.

A second type of artifact involves the other extreme when the absorbance is large. When the absorbance approaches 2.0 the accuracy of the measurement is compromised. The light intensity reaching the detector is quite low under these conditions. The detector response is not linear in this extreme. The absorption bands with A > 1.5 can appear distorted or even cut off. These is no way to rectify data obtained under these conditions. Instead, one must ensure that all concentrations used in an experiment give absorbance A < 1.5. One can use shorter path length cells if the absorbance is too high. However, the practical limit is approximately 100 microns for cell thickness. Most absorbance cells are between 0.1 – 1.0 cm.

The Beer-Lambert Law

The intensity of absorption is experimentally related to the integrated extinction coefficient. The extinction coefficient, $\varepsilon(\nu)$ is tabulated in many sources, including the CRC Handbook. The extinction coefficient is related to the absorbance according to Beer's law.

$$A = \varepsilon c \ell$$

where C is the concentration and ℓ is the path length. Note that the units of ε are $M^{-1}cm^{-1}$. A is unitless. The transmitted intensity, I, is related to the incident intensity, I_0, by

$$I = I_0 10^{-A}$$

In this section we merely point out that the extinction coefficient must be related to the overall absorption band area (i.e. the intensity). The Franck-Condon factor determines the shape of the band and the square of the electron coupling (transition dipole moment) determines the magnitude of the absorption band area.

Experimental Design of Absorption Spectrometers

There are two common configurations of absorption spectrometers, scanning and photodiode array. A scanning instrument uses a diffraction grating to disperse the radiation and then a slit to select a narrow bandwidth from the radiation. As the diffraction grating is rotated the wavelength of the light that passes through the list is changed. Thus, an absorption spectrum is measurement point-by-point in a scanning mode. A photodiode array uses a collection of detectors to simultaneously measure all wavelengths in the sample.

In both methods, the limit of the possible wavelengths is determined by light sources and detector sensitivity. The most common detector in an absorption spectrometer is a silicon photodiode. Since the band gap of silicon is approximately 1.2 eV that represents the lowest energy that can be measured using a silicon photodiode. The sensitivity of silicon also decreases at energy higher than 3.1 eV. In wavelength units of nanometers the useful range of a Si photodiode is 400-1000 nm. To measure ultraviolet wavelengths one needs to use a photomultiplier tube.

In order to obtain a spectrum of the molecule of interest without interference from solvent or other species the procedure involves making two measurements, first a reference spectrum of the cuvette and solvent, but without the molecule and second, a sample spectrum in an identical cuvette and solvent, but containing the molecule of interest. The goal is to ratio the parts of the signal that do not belong to the molecule. These procedure is represented in figure 1. The figure shows that that sample intensity is divided by the reference intensity. This gives the transmission

$$T = \frac{I}{I_0}\ 100\%$$

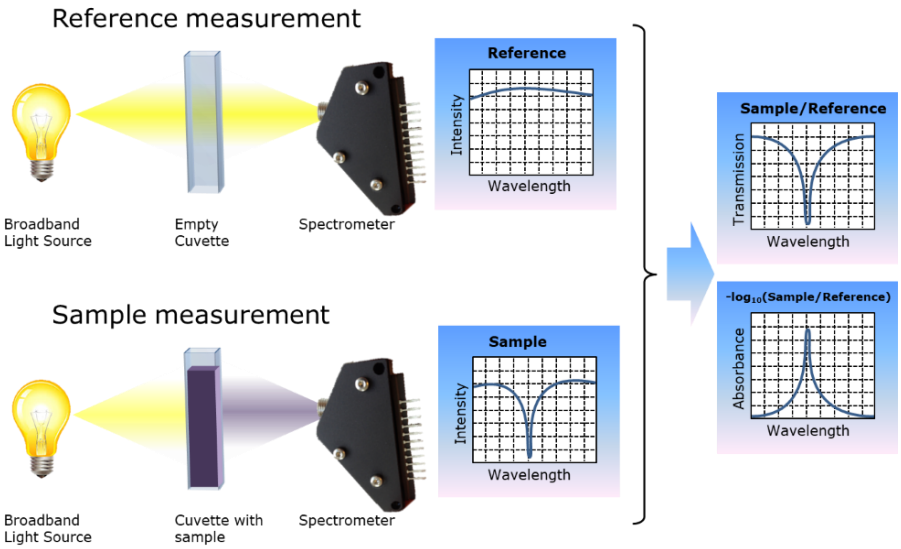


Figure 1. Representation of the reference and sample measurements in an absorption spectrometer.

The intensity ratio is converted to absorbance.

$$A = -log_{10}\left(\frac{I}{I_0}\right)$$

Figure 1 shows the two intensities. I_0 is the reference intensity and I is the sample intensity. Our discussion has not mentioned wavelength yet, but these intensities are both dependent upon wavelength. Thus, the ratio of I/I_0 is a ratio of spectra as indicated in figure 1.

Practical Thinking in Absorbance Processes

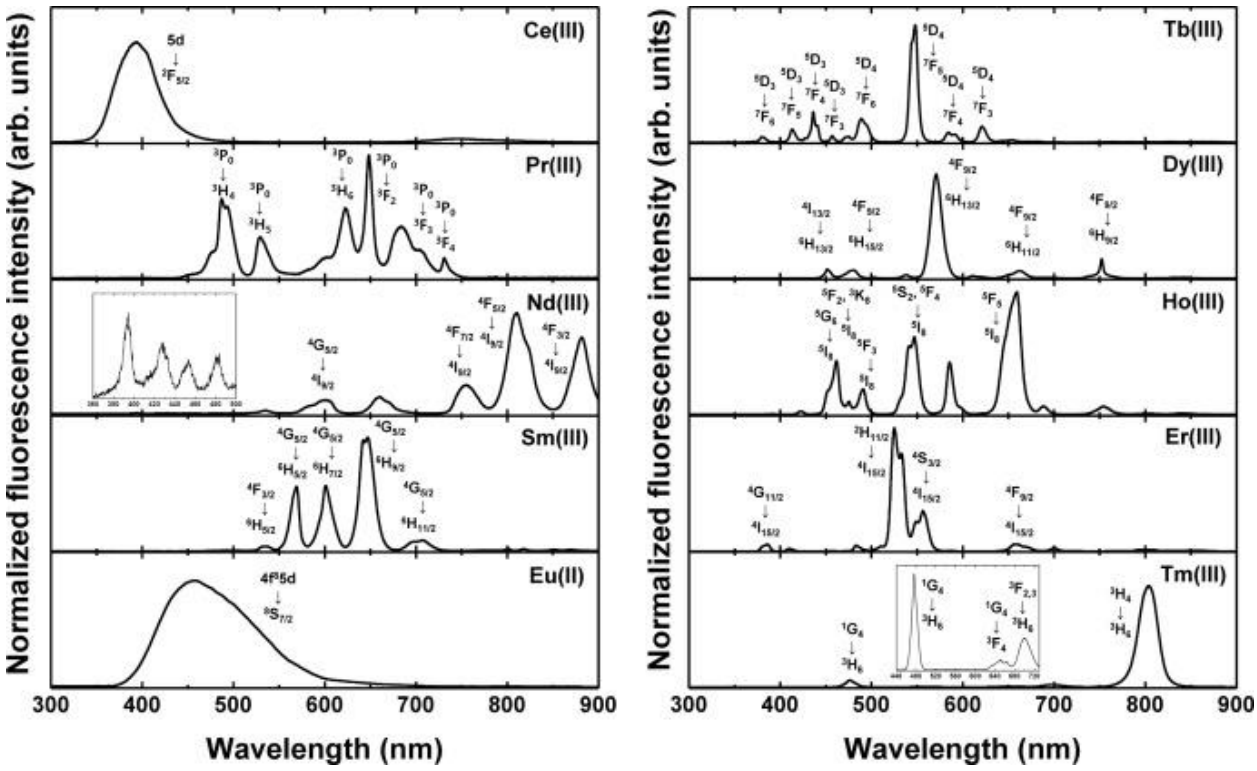
You might think that it is impossible to calculate logarithms in your head. But, consider the following. An absorbance of A = 0.3 corresponds almost exactly 50% transmittance. There is a useful filter in spectroscopy known as a neutral density filter that absorbs all wavelengths equally. A ND50 filter has 50% transmittance and therefore an absorbance of A = 0.3. A ND25 filter has 25% transmittance and that corresponds to A = 0.6. You multiply the transmittance and add the absorbance. 12.5% transmittance corresponds to A = 0.9. Of course, you can estimate in your head that 10% transmittance corresponds to A = 1.0. This continues. A ND5 filter has 5% transmittance and corresponds to 50% of 10% or A = 1.3. A 1% transmitting sample has A = 2.0.

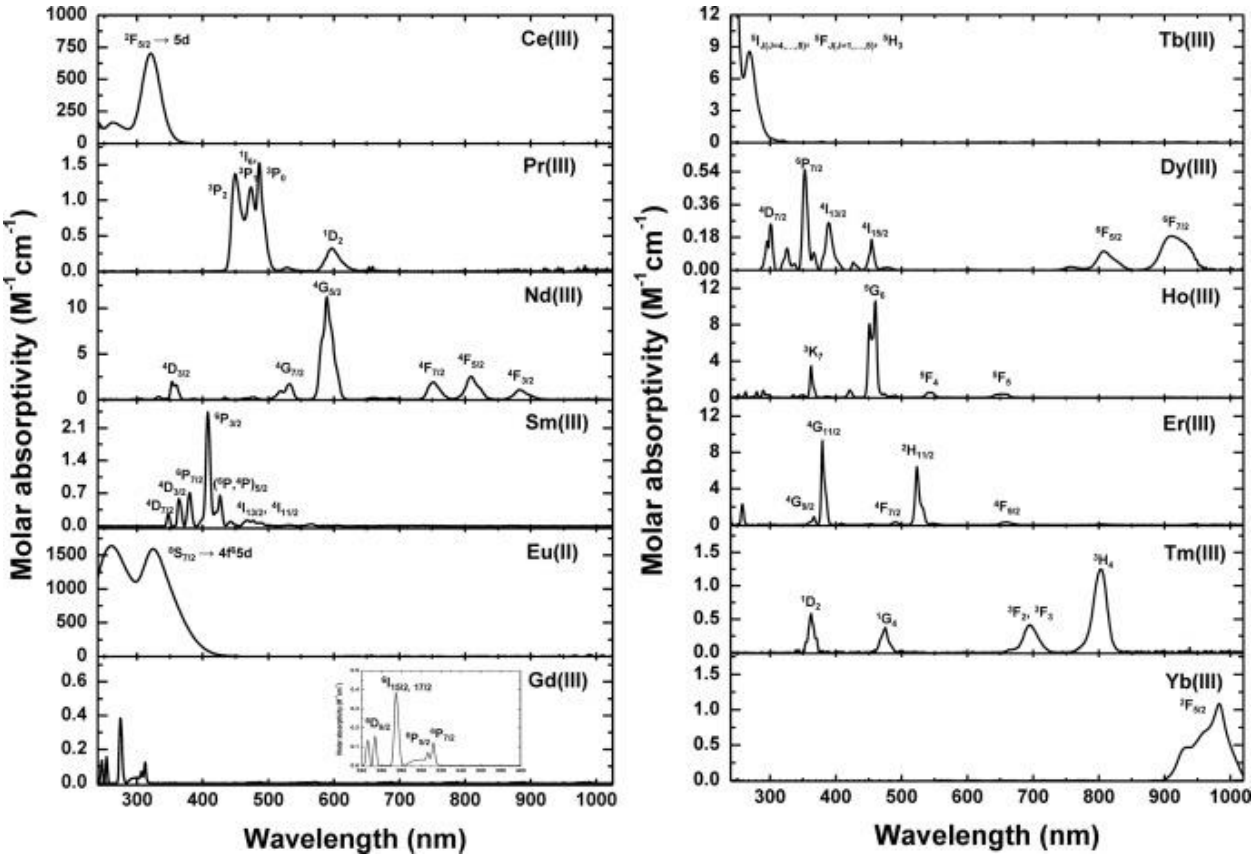
These facts are useful to keep in mind when planning an absorption experiment. You want the highest possible signal-to-noise ratio for any measurement. The signal should be greater than A = 0.1 for that reason. The noise from the detected is usually in the range of 0.005 on most inexpensive photodiode array spectrophotometers. If the absorbance of the sample is too small then the signal is too small. But the absorbance can be too high as well. If the absorbance approaches A = 2.0 then you are in the range where only 1% of the incident light is reaching the detector. This is such a low light level that there is also a small signal-to-noise ratio. The optimal absorbance range is from A = 0.3 to 0.8. It is safe to work from A = 0.1 to 1.4 or so. Sometimes it is necessary to go outside these ranges because of sample constraints. It is permissible to do so, but one must always be aware of the possible loss of accuracy. Think about alternatives. One alternative for high absorbing samples is a shorter path length. There are cuvettes with 0.4 cm, 0.1 cm and even down to 100-micron path length. The latter is difficult to use, but the others are standard.

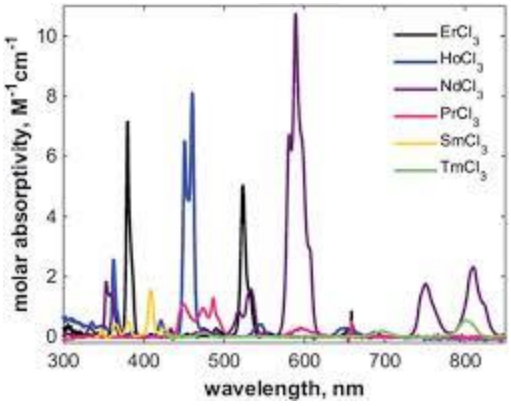
Lanthanides as Spectroscopic Probes

The intensities of absorption spectral bands of lanthanides can be explained using Judd-Ofelt theory. Formally, these transitions are La Porte forbidden since f to f involves a transition between two orbitals with the same angular momentum quantum number.

The work of Kim et al. in Ref. 1 describes absorption and fluorescence spectra of a series of lanthanide elements (La, Ce, ···, Yb, Lu) in high temperature molten LiCl–KCl eutectic. These were compared to aqueous spectra providing information on assignments in both solvents.


Normalized fluorescence intensity (arb. units)
Wavelength (nm)
Ce(III)
Pr(III)
Nd(III)
Sm(III)
Eu(II)
Tb(III)
Dy(III)
Ho(III)
Er(III)
Tm(III)
300
400
500
600
700
800
900

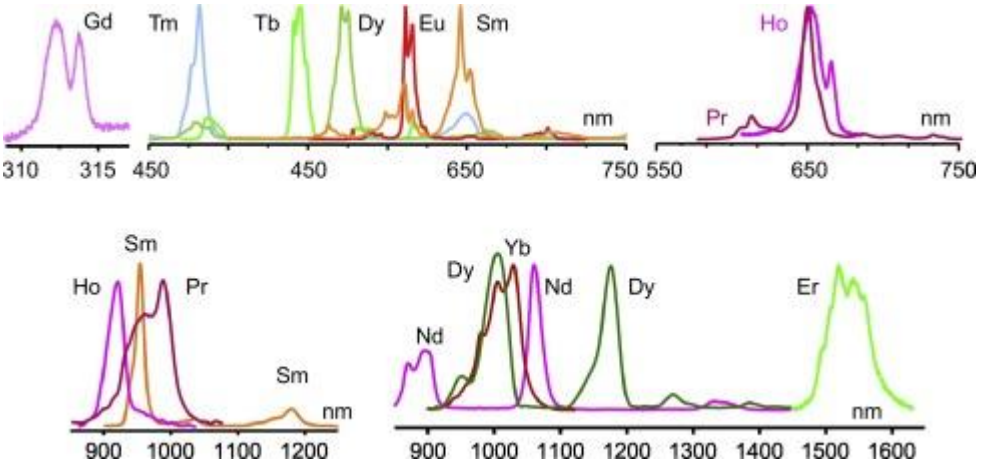




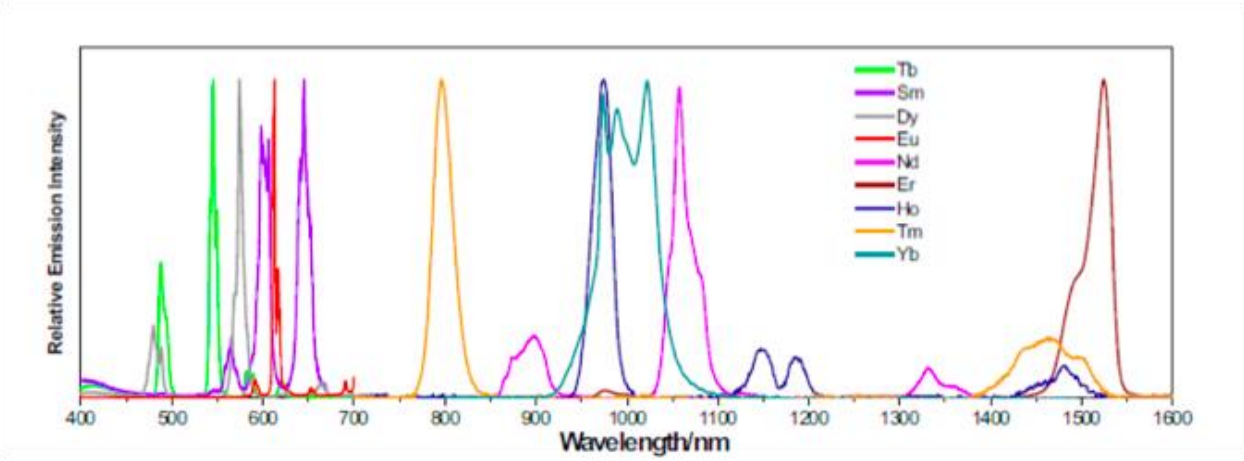
Sensitization as a Strategy to Enhance Lanthanide Emission

The sharp luminescence bands of lanthanides are shown below. Their insensitivity to the environment makes them attractive as imaging agents. Because the f-f transitions of lanthanides

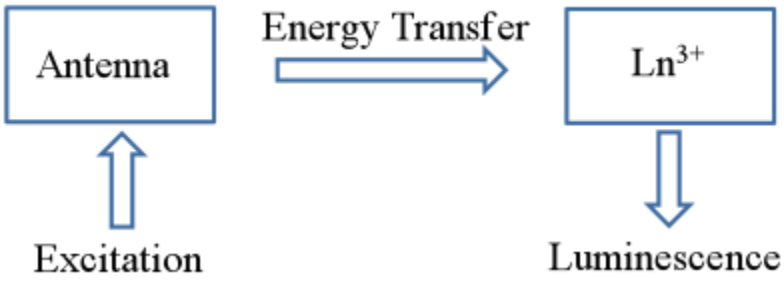
are forbidden, both their absorption and emission strengths are weak. Sensitization is one method used to overcome this limitation.



The spectra above are from Ref. 2.



The strategy that has been investigated by many research groups uses the antenna concept. A sensitizer that acts as a funnel for the light energy could be a molecule (ligand) or a semi-conductors. Research at the University of Pittburgh, Department of Chemistry indicates that radiation can be captured and transfered non-radiatively to lanthanide ions sufficient close to a sensitizer. CdSe nanoparticles have been used as an antenna to sensitize Tb^{3+} luminescence. More research research has explored ZnS nanoparticles, which both better sensitizers and less toxic, compared to the constituents in CdSe nanoparticles.



Lanthanide Sensitization in II-VI Semiconductor Materials
Group Members: Prasun Mukherjee, Daniel N. Lamont, Robin Sloan

1. Kim, B. Y. and Yun, J.I. *J. Luminescence*, **2016**, *178*, 331-339
2. Chengelis, D. A.; Yingling, A. M.; Badger, P. D.; Shade, C. M.; Petoud, S. *J. Am. Chem. Soc.* **2005**, *127*, 16752-16753.